Semi-field evaluation of a granulovirus and *Bacillus thuringiensis* ssp. *kurstaki* for season-long control of the potato tuber moth, *Phthorimaea operculella*

Steven P. Arthurs[1], Lawrence A. Lacey[2]*, Jonathan N. Pruneda[2] & Silvia I. Rondon[3]

[1]UF-IFAS, Mid-Florida Research and Education Center, 2725 S. Binion Road, Apopka, FL 32703, USA, [2]USDA-ARS, Yakima Agricultural Research Laboratory, Wapato, WA 98951, USA, and [3]Hermiston Agricultural Research and Extension Center, Hermiston, OR 97838, USA

Abstract

There are few insecticidal options for potato tuber moth (PTM), *Phthorimaea operculella* Zeller (Lepidoptera: Gelechiidae), control late in the growing season. We evaluated the PTM granulovirus (PoGV) and *Bacillus thuringiensis* Berliner ssp. *kurstaki* (Btk) for season-long control of PTM on potato foliage in 2006 and 2007. Compared to untreated controls, 10 weekly applications of PoGV (10^{13} occlusion bodies/ha) reduced PTM populations in replicated 1-m^3 field cages by 86–96% on pre-harvest foliage and 90–97% on tubers added to cages shortly before harvest. Infection rates of 82–95% of L4 larvae by PoGV were noted within individual larval cohorts. Equivalently timed Btk treatments (1.12 kg product/ha) were significantly less effective at population suppression, with a 36–76% reduction in larvae recovered from tubers added to cages. A PoGV/Btk alternation was significantly more effective than Btk alone and as effective as PoGV in 2007, but not in 2006. There was some evidence that reduced rate PoGV treatments (10% rate or 50% application frequency) were less effective than the standard program. There were no treatment effects on percentage of tubers growing in the ground that were infested at harvest, which remained comparatively low at ≤8.1%. Bioassays were conducted to evaluate the residual activities of foliar deposits. Early-season applications were highly effective for the first 24 h (≥93% mortality) with a steady decline in activity over 10 days. A second application, applied later in the season, showed similar patterns, although in this case Btk was less persistent than PoGV, whereas both agents provided significant larval mortality compared with controls over 14 days. Both PoGV and Btk provide alternatives to manage field infestations of PTM prior to harvest, thus reducing the risk of tuber infestations in storage.

Introduction

Native to tropical mountain regions of South America, the potato tuber moth (PTM), *Phthorimaea operculella* Zeller (Lepidoptera: Gelechiidae), is now a cosmopolitan pest of potato, *Solanum tuberosum* L. (Solanaceae), and other solanaceous crops throughout the tropics and subtropics (Radcliffe, 1982; Trivedi & Rajagopal, 1992). Economically

significant outbreaks of PTM have also been reported in more temperate zones including New Zealand (Cameron et al., 2002), Australia (Symington, 2003), southwestern Europe (Povolný & Hula, 2004), and most recently the Pacific Northwest of the USA (Jensen et al., 2005). During the growing season, PTM larvae mine leaves, stems, and petioles, but during plant senescence will readily infest tubers. Moths may also enter cracks in the ground to oviposit on or close to tubers (Radcliffe, 1982). In addition to direct damage, transfer of field-infested tubers to storage may facilitate damage by secondary pests and diseases, and severely reduce the quality and market value of a crop. In rustic non-refrigerated stores, PTM

*Correspondence: Lawrence A. Lacey, USDA-ARS, Yakima Agricultural Research Laboratory, 5230 Konnowac Pass Rd., Wapato, WA 98951, USA. E-mail: lerry.lacey@ars.usda.gov

infestations may destroy an entire crop within 2–4 months (Von Arx et al., 1987).

Current options for control of PTM prior to harvest include several broad spectrum insecticides and various cultural controls that reduce the risk of tuber infestations. Although potato plants can withstand significant foliar damage without yield loss (Shields & Wyman, 1984), there is often zero tolerance for pest infestation in the fresh market. Thus, the most economically significant damage typically occurs shortly before harvest, especially following vine desiccation, when the risk of tuber infestation is highest. Because pre-harvest intervals limit the use of broad spectrum pesticides late season (e.g., 14 days for methamidophos), alternative options to control PTM would be beneficial by providing options for late-season and post-harvest control, as well as tools for insecticide-resistance management strategies and organic production.

A granulovirus (Baculoviridae) associated with PTM (PoGV) has been isolated in the tropics and subtropics in various parts of the world (summarized by Zeddam et al., 1999). PoGV has also been documented in California (Hunter et al., 1975), but not in Washington state or elsewhere in North America. Previous studies have demonstrated the potential of PoGV to control PTM under both field and storage conditions (Reed & Springett, 1971; Von Arx & Gebhardt, 1990; Ben Salah & Aalbu, 1992; Das et al., 1998; Hanafi, 2005). In Peru, the International Potato Centre (CIP) has developed a dry formulation of PoGV that can be mixed with inert carriers such as talc (magnesium silicate) as a method to protect tubers in rustic stores in several countries (Lagnaoui et al., 1997).

The bacterium *Bacillus thuringiensis* Berliner (Bt), with a long history of commercial development against Lepidoptera, is another promising pathogen for the control of PTM. Laboratory tests have identified *B. thuringiensis* ssp. *kurstaki* (Btk) as one of the most virulent strains against PTM (Salama et al., 1995a), and various commercial preparations have been tested under both field and storage conditions (Von Arx & Gebhardt, 1990; Broza & Sneh, 1994; Salama et al., 1995b; Kroschel & Koch, 1996; Das et al., 1998). To our knowledge, there are no reports of microbial control of PTM in North America or Europe. In this article, we evaluated different treatments of PoGV and Btk for season-long control of PTM on potato foliage under growing conditions of central Washington state.

Materials and methods

Potato tuber moth colony

A PTM colony derived from a field population collected at the Oregon State University Hermiston Agricultural Research and Experiment Station in 2004 was maintained on potato cv. 'Russet Burbank' at 27 °C, 25% r.h., and L16:D8. In the rearing procedure, washed tubers were placed in 7.6-l Tupperware containers containing medium grade sand as a pupation substrate, and infested with eggs laid on filter paper within 24 h of hatching. Pupae were collected 16 days later and separated from sand using a 1-mm sieve. Silken cocoon casings were dissolved in 1.25% NaClO to facilitate emergence. Healthy pupae were rinsed and moved to 0.47-l oviposition cups containing muslin tops (approximately 200 pupae/cup). Eclosing adults were fed with 15% (wt/vol) sugar solution on cotton wicks and produced eggs on moistened 9-cm-diameter filter papers placed on top of cups. Eggs were collected at 24-h interval for 5 days of oviposition and used to start new colonies.

Source of PTM granulovirus and *Bacillus thuringiensis* Berliner ssp. *kurstaki*

The PoGV isolate used in our studies was derived from a dry formulation produced by CIP (1992) in Lima, Peru, and imported from Ecuador under permit no. 29878. For the in vivo virus production, PTM egg sheets were dipped in suspensions containing one virus-infected fourth instar equivalent (LE)/ml, while tubers were dipped at the rate of 1 LE/100 ml, and air dried before infestation. Infected larvae were collected 21–28 days post-infestation and frozen until use in further virus production or for experiments. For virus quantification in our experiments, 300 infected fourth instars were macerated in 50 ml sterile water with a glass tissue grinder. The homogenate was vacuum filtered through four layers of organdy cloth. Two 5-ml aliquots were taken for purification using the sucrose gradient method outlined by Sporleder et al. (2005) and quantification of occlusion body (OB) density using dark field microscopy and a thin (10 μm) Petroff-Hausser counting chamber (Electron Microscopy Sciences, Hatfield, PA, USA). The remainder (unpurified material) was frozen (–70 °C) in aliquots for future field use. New quantities of virus were produced every 3 months. A commercial strain of Btk formulated as a wettable powder containing 85% of solids, spores, and toxins (Deliver®; Certis USA, Columbia, MD, USA) was used in all tests.

Field site and experimental cages

Field studies were conducted in 2006 and 2007 at the USDA-ARS research farm near Moxee, WA, USA. The study site was a 0.2-ha potato field cv. Russet Burbank planted early May (different location each year) with 30 cm spacing (10 cm hill depth) and irrigated and cultivated according to regional practices. At vegetative growth stage II (Rowe, 1993), 35 field cages were erected, each separated by at least 2 m. Cages consisted of a 1-m³ wooden frame dug into the ground around three plants. The frame was enclosed with

an acrylic mesh cover that allowed 85% direct light transmission ('Econet T', 0.15 × 0.35 mm hole size; US Global Resources, Seattle, WA, USA) that was secured in the ground. A large removable panel attached by Velcro provided access for spraying and sampling. Potato tuber moth infestations were established in each cage by releasing 30 pupae per cage (50:50 sex ratio). To extend oviposition periods, releases were made twice, that is, 20 and 10 pupae at 42 and 50–52 days post-planting, respectively. An elevated release platform was designed to protect emerging pupae (>90% emergence was confirmed). Natural inflorescences on some plants may have provided a food source for moths.

Shade temperature, relative humidity, and ambient solar intensity were monitored with a Hobo H8 Pro Series data logger (Onset Computer, Pocasset, MA, USA) and an LI-400 data logger fitted with a pyranometer (Li-Cor, Lincoln, NE, USA). Conditions remained sunny and dry; only 0.33 cm (2006) and 1.24 cm (2007) rain fell throughout the study. Daily maximum and minimum temperatures (inside cages) were 30.2 °C (range 21.0–40.1) and 11.3 °C (3.7–19.8) in 2006, and 29.1 °C (20.2–38.8) and 11.6 °C (1.6–22.1) in 2007. Maximum solar radiation intensity (outside cages in July and August) remained similar ($\approx$ 1000 W m^{-2}) in both years.

Experimental treatments

Granulovirus and Btk were applied weekly 10 times throughout the growing season; starting 10–12 days post-infestation (3% egg hatch of the F_1 generation as monitored inside surplus cages) and continuing throughout the F_2 larval generation. Treatments comprised PoGV at 10^{13} OB/ha (= 435 LE based on our virus quantification), Btk at 1.12 kg/ha, PoGV/Btk rotation (weekly alternation at the same rates) and controls (water + wetting agent). We also evaluated reduced rate PoGV treatments (a priori contrasts), comprising 10^{12} OB/ha applied weekly or 10^{13} OB/ha at 2-week intervals. There were five replicate cages per treatment allocated as a randomized complete block (within row) design. In line with commercial practices, at the end of the study diquat dibromide (Reglone®; Syngenta Crop Protection, Basel, Switzerland) was applied at 1.8 l/ha to all cages to desiccate potato vines.

All treatments were applied at 500 l/ha using a CO_2 pressurized backpack sprayer (10 l capacity, Model T; R & D Sprayers, Opelousas, LA, USA). The spray lance consisted of a perpendicular boom (46 cm) fitted with three flat fan nozzles (TeeJet 8002VS®; Wheaton, IL, USA) and operated at 206 kPa. The wetting agent Silwet L77® (Silicone-polyether copolymer; Loveland Industries, Greeley, CO, USA) was added at 0.05% to all treatments and the spray tank agitated to prevent settling. The chemical standard

methamidophos (Monitor®; Bayer Crop Science, Monheim, Germany) was applied at 1.75 l/ha (diluted at 500 l/ha) at 14-day intervals (twice per larval generation) using a Solo® hand pressurized backpack sprayer (15-l capacity; Newport News, VA, USA) fitted with a single cone nozzle. All treatments started on the same day and applications were made before 09:00 hours during calm wind conditions.

Releases of the aphid parasitoid *Aphidius ervi* Haliday (Rincon-Vitova Insectaries, Ventura, CA, USA) and 1–2 applications of selective aphidicide pyrometrizine (Fulfill; Syngenta) at 192 g/ha were used against outbreaks of *Myzus persicae* Sulzer and *Macrosiphum euphorbiae* Thomas, inside some cages (all cages were treated). Nevertheless, plants in some cages were destroyed by high aphid populations (especially during the 2006 pre-harvest); these cages were excluded in our data.

Data collection

Foliage samples (1 stem per plant; that is, 3 stems per cage) were taken at the end of the first and second larval generations (vegetative stage IV and V or 38–40 and 77–79 days post-infestation, respectively). Plant injury (% mined leaves) and the developmental stage of live larvae dissected from leaf mines were recorded in the laboratory (samples were maintained at 4 °C until processing). In PoGV treatments, the proportion of infected fourth instars was also noted (younger larvae were reared on tubers in the laboratory to confirm infection status).

Following application of the vine desiccant, 12 tubers (dug up from adjacent plants and washed) were placed inside each cage to capture larvae exiting dying foliage. Tubers were collected after 7–10 days and incubated in 20-l buckets containing sand to quantify the number of fourth-instar PoGV-infected and healthy larvae that emerged. Plants inside cages were dug up a week later (after dismantling cages) to determine any natural infestation of tubers in the ground. Prior to excavation, a short-acting pyrethrin (Pyrenone®; Bayer Crop Science) was sprayed to kill remaining moths to minimize infestation of surrounding plants. Tubers were washed and thinly sliced for evaluation.

Residual activity study

The residual activities of PoGV and Btk were evaluated in a different section of the potato field described above. Applications to uncaged plants were made on 12 June 2006 and 13 August 2007, 34 and 96 days post-planting, respectively. Experimental groups were treated at concentrations concordant to those used in the cage study. On each occasion, six leaf samples per treatment were collected at 0, 1, 3, 5, 7, 10, and 14 days following application for bioassay in the laboratory. On sampling days, leaflets were removed at the petiole base mid-way up

sprayed plants and the cut section placed in water in a 15-ml conical vial. The leaflet was housed inside a 14-cm diameter Petri dish, with a hole to accommodate the vial. For the bioassay, the upper surface was infested with 10 neonates (<4 h old). Petri dishes were sealed using Parafilm® and incubated at 25 °C. After 48 h, leaflets were removed, examined at 10× for larval mines, and placed on top of single tuber in a 0.47-l cup with sand for larval development. Healthy pupae or emerged moths were evaluated after 28 days at 25 °C.

Data analysis

In the field cage study, treatment effects were compared using univariate analysis of variance (ANOVA) with significant main effects further separated using Fisher's least significant difference for multiple comparisons. The numbers of leaf mines or larvae were $\sqrt{x}$ transformed, and the proportion of leaf mines or larvae pupating were arcsine $\sqrt{x}$-transformed before analysis (SAS; SAS Institute, Cary, NC, USA). Rates of infection among different PoGV treatments (these data were pooled among cages due to the low numbers in some cases) were compared using categorical χ^2 analysis (SAS Institute, 2001). In the residue study, effects of treatment and time on the number of leaf mines and larval mortality were assessed using a repeated measures ANOVA, having three treatments (untreated, PoGV, and Btk) and seven time intervals (Proc MIXED; SAS Institute, 2001). The AR(1) covariance structure was used to account for the correlations among the repeated levels (time). In the event of a significant treatment*time interaction, the SLICE command was used to compare treatments for each time interval separately.

Results

Field cage study

In both years, PTM populations established in field cages progressed through two generations prior to the pre-harvest sampling. The F_1 adult generation was first observed at 31 and 46 days post initial infestation, in 2006 and 2007, respectively, and remained active for several weeks. Prolonged egg hatch and high densities of the F_2 larval generation were observed, illustrated by the 98 and 83% of mined leaves found in the pre-harvest sample in control cages in 2006 and 2007, respectively (Tables 1 and 2).

Weekly treatment with PoGV (10^{13} OB/ha) did not reduce initial damage (% mined leaves) or mid-season larval abundance compared to controls (Tables 1 and 2). However, a high proportion of F_1 larvae ($\geq$90%) reared out in the laboratory were confirmed virus-infected (Table 3). Infected larvae were identified by their opaque milky white color, sluggish behavior, and, although usually completing larval development, were never observed to pupate and hence reproduce. Control in the PoGV treatment is thus illustrated by the reductions in mined leaves (76.3% in 2006 and 73.3% in 2007), F_2 larvae recovered from both foliage (96.3% in 2006 and 85.8% in 2007), and artificially added tubers (97.4% in 2006 and 90.3% in 2007) compared to untreated controls in the pre-harvest sample (Tables 1 and 2). In terms of F_2 larvae recovered from tubers, weekly treatment of PoGV at 10^{13} OB/ha was as effective as four

Table 1 Evaluation of granulovirus (PoGV) and *Bacillus thuringiensis* ssp. *kurstaki* for season-long control of potato tuber moth (PTM) in 1-m³ field cages, at USDA-ARS Moxee Research Farm, 2006

| Treatments | Rate/ha | No. applications | Mid-season (F_1 generation)[1] | | Pre-harvest (F_2 generation)[1] | | |
			% mined leaves	PTM/100 leaves	% mined leaves	PTM/100 leaves	PTM/tubers
Untreated control	N/A	10	37.3 (4.5)ab	15.1 (3.6)ab	97.9 (2.1)a	236.5 (36.5)a	1119 (38)a
Methamidophos	1.75 l	4	3.7 (0.9)c	0.5 (0.2)c	4.2 (1.8)c	2.2 (1.1)d	59 (22)cd
PoGV	10^{13} OB	10	45.8 (4.6)a	43.3 (14.5)a	23.2 (7.8)b	8.7 (1.3)bc	29 (17)d
Btk	1.12 kg	10	23.1 (7.4)b	11.6 (3.9)b	23.7 (2.0)b	19.9 (4.5)b	266 (48)b
PoGV/Btk[2]	10^{13} OB/1.12 kg	10	26.6 (5.7)b	15.9 (2.7)ab	26.2 (5.3)b	6.7 (0.8)c	83 (16)c
Reduced rate PoGV treatments (a priori contrasts)							
PoGV	10^{13} OB	10	45.8 (4.6)	43.3 (14.5)	23.2 (7.8)	8.7 (1.3)b	29 (17)b
PoGV (low rate)	10^{12} OB	10	40.7 (4.1)	28.9 (8.9)	47.4 (13.2)	35.2 (4.5)a	121 (45)a
PoGV (low frequency)	10^{13} OB	5	31.4 (5.3)	24.2 (8.1)	23.0 (2.7)	25.0 (17.9)ab	157 (39)a

Different letters within a column indicate significant differences (Fisher's LSD: P<0.05) following significant effects as indicated by ANOVA.
[1]Mean (SEM) from a minimum of 100 leaves/cage, including tubers added to cages.
[2]Alternating weekly.

Table 2 Evaluation of granulovirus (PoGV) and *Bacillus thuringiensis* ssp. *kurstaki* for season-long control of potato tuber moth (PTM) in 1-m³ field cages, at USDA-ARS Moxee Research Farm, 2007

| Treatments | Rate/ha | No. applications | Mid-season (F_1 generation)[1] | | Pre-harvest (F_2 generation)[1] | | |
			% mined leaves	PTM/100 leaves	% mined leaves	PTM/100 leaves	PTM/tubers
Untreated control	N/A	10	36.1 (3.7)a	23.2 (4.6)a	83.1 (4.9)a	363.0 (93.2)a	657 (91)a
Methamidophos	1.75 l	4	1.0 (0.2)c	0.2 (0.1)b	1.4 (0.5)c	0.5 (0.2)c	22 (9)d
PoGV	10^{13} OB	10	34.1 (7.5)a	28.2 (8.9)a	22.2 (10.9)b	51.6 (46.3)b	64 (27)cd
Btk	1.12 kg	10	26.0 (2.2)ab	14.5 (2.7)a	16.4 (3.9)bc	18.1 (3.3)b	421 (105)b
PoGV/Btk[2]	10^{13} OB/1.12 kg	10	21.6 (3.2)b	13.9 (3.5)a	14.8 (2.9)bc	8.7 (1.0)b	68 (9)c
Reduced rate PoGV treatments (a priori contrasts)							
PoGV	10^{13} OB	10	34.1 (7.5)	28.2 (8.9)	22.2 (10.9)	51.6 (46.3)	63.5 (27.3)c
PoGV (low rate)	10^{12} OB	10	35.1 (5.6)	25.7 (3.6)	38.9 (6.7)	64.2 (7.7)	307.5 (29.2)a
PoGV (low frequency)	10^{13} OB	5	34.6 (10.2)	22.6 (9.0)	37.0 (7.3)	113.2 (68.1)	176.0 (32.0)b

Different letters within a column indicate significant differences (Fisher's LSD: $P<0.05$) following significant effects as indicated by ANOVA.

[1]Mean (SEM) from a minimum of 100 leaves/cage, including tubers added to cages.

[2]Alternating weekly.

applications of methamidophos. Levels of PoGV-infection among fourth instars reared from foliage and tubers remained high in the F_2 generation (≥82%), which would have provided additional control of the F_3 generation (Table 3).

The Btk treatment provided equivalent or better foliar protection (mines and larvae recovered on leaves) compared to PoGV (but not methamidophos), but were less effective at population control overall, illustrated by only a 76.2% (2006) and 35.9% (2007) reduction in F_2 larvae infesting artificially added tubers pre-harvest compared to controls (Tables 1 and 2). The PoGV/Btk rotation was more effective than Btk in both years, but less effective than PoGV in 2006 but not in 2007.

There was evidence that reduced rate PoGV treatments were less effective, illustrated by pre-harvest data on foliage (2006) and tubers (both years). Interestingly, there were no significant differences in infection rates obtained from

fourth instars reared from foliage treated with reduced rates of PoGV in 2006 (χ^2≤2.2, d.f. = 2, P≥0.09). In 2007, proportionally fewer infected larvae were recovered from the low rate (10^{12} OB/ha), but not low frequency treatment at both mid season ($\chi^2 = 4.9$, d.f. = 2, P<0.0001) and pre-harvest ($\chi^2 = 6.3$, d.f. = 2, P<0.0001) (Table 3).

Infestation of tubers growing in the ground inside cages (i.e., dug up at harvest) remained comparatively low, namely, ≤8.1% in 2006 (n = 1152) and ≤2.9% in 2007 (n = 1481). There were no significant effects of insecticidal treatment in either year ($F_{6,34} = 2.4$, P = 0.06 and $F_{6,31} = 1.1$, P = 0.38, respectively).

Residual activity of PTM granulovirus and *Bacillus thuringiensis* Berliner ssp. *kurstaki* under field conditions

Repeated measures ANOVA revealed treatment*sample period interactions for both the number of leaf mines at 48 h and PTM reared from our laboratory bioassays on

Table 3 Average granulovirus (PoGV) infection rates of reared fourth-instar potato tuber moth collected mid-season (F_1 generation) and pre-harvest (F_2) in field cages treated with PoGV over 2 years

| Treatment[1] | 2006[2] | | 2007 | |
	Mid-season	Pre-harvest	Mid-season	Pre-harvest
PoGV	89.8 (93)	81.9 (362)	95.4 (256)	92.9 (627)
PoGV (low rate)	83.5 (178)	83.6 (1122)	78.4 (223)	85.8 (2774)
PoGV (low frequency)	72.7 (76)	84.2 (722)	94.6 (216)	93.6 (1646)

[1]See Tables 1 and 2.

[2]Mean (sample n) pooled among five cages; mid-season samples collected from foliage, pre-harvest samples collected on foliage and tubers added to cages; see text for χ^2-analysis.
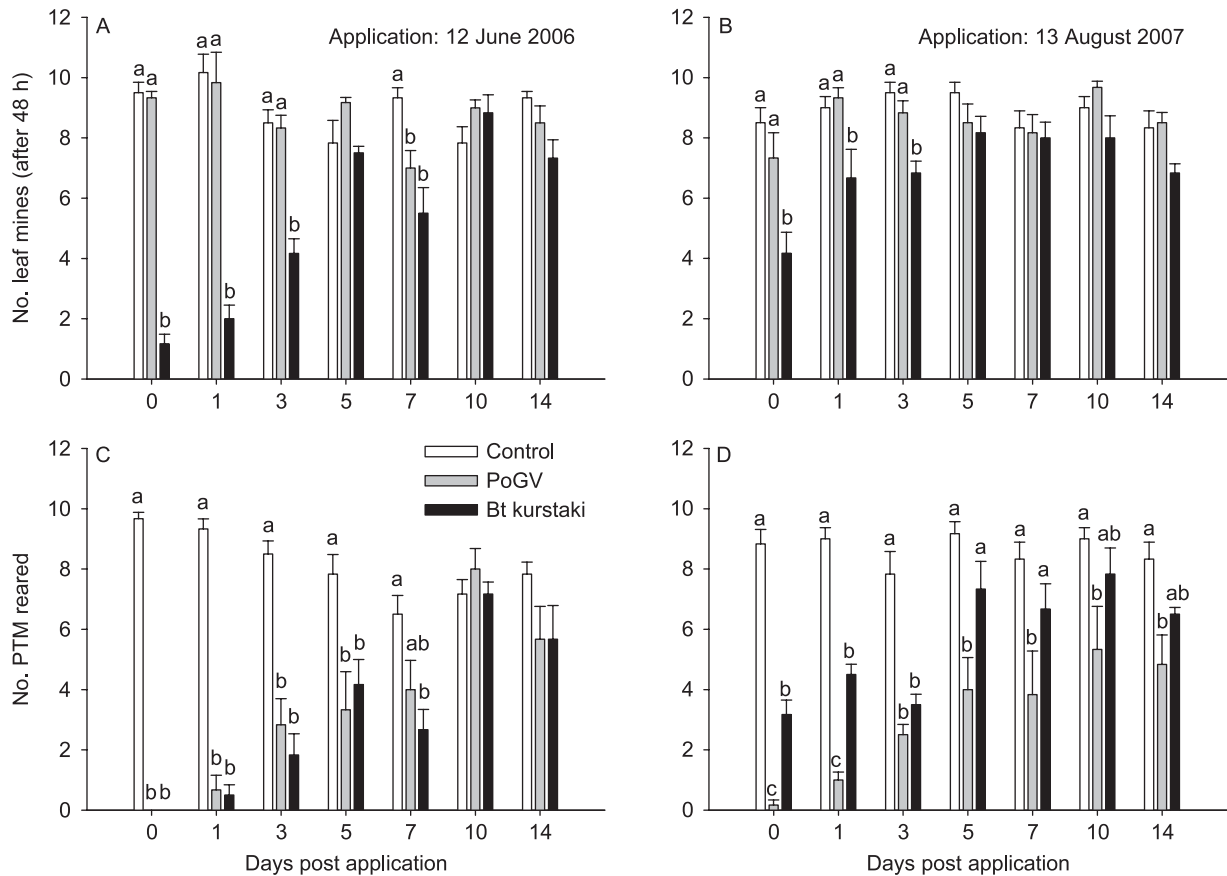
Figure 1 Number of leaf mines and potato tuber moths reared from leaves with residues of potato foliage sprayed with *Phthorimaea operculella* granulovirus (PoGV, 10^{13} OB/ha) and *Bacillus thuringiensis* ssp. *kurstaki* (Bt kurstaki, 1.12 kg/ha). Ten neonates were exposed to individual leaf samples and transferred to potatoes after 48 h for rearing. Data shown for treatments applied on two dates. Letters denote significant treatment effects at equivalent intervals post spraying.

aged residues of PoGV and Btk applied on 12 June ($F_{12,90} \geq 15.7$, P<0.0001) and 13 August ($F_{12,90} \geq 2.3$, P<0.05). Fewer mines were recorded on foliage up to 3 days following treatment with Btk compared to PoGV and control foliage for both years (Figure 1A,B). In both years, fewer PTM pupated following exposure to foliage with residues of Btk and PoGV, compared to the control, up to 14 days after application (Figure 1C,D). The estimated half lives calculated from the regression line describing larval mortality over the periods where significant treatment effects were detected following the June (2006) and August (2007) applications were 4.1 and 5.1 days for PoGV and 4.2 and 3.9 days for Btk, respectively.

Discussion

In our field cages, weekly PoGV applications (10^{13} OB/ha) infected ≥82% fourth instars and reduced PTM

populations 86–96% on pre-harvest foliage and 90–97% on tubers added to cages shortly before harvest, confirming the effectiveness of the virus under growing conditions in central Washington. *Bacillus thuringiensis* ssp. *kurstaki* within label rates was as effective as PoGV in terms of foliar infestations, but only provided a 36–76% reduction in larvae recovered from tubers added to cages. In the latter case, it is likely that moths (late F_2 or early F_3) directly ovipositing on these tubers (after foliage had died) contributed additional larvae in Btk treatments. In contrast, the correspondingly fewer adults expected in the PoGV treatments (based on larval infection rates) would explain reduced tuber infestations observed in both years. We demonstrated the potential to alternate PoGV with Btk. Although PoGV is only known to infect PTM and closely related gelechiids, combined use with Btk would provide a strategy for resistance management for both PoGV (Briese & Mende, 1983) and Bt (Tabashnik, 1994).

However, recent reports on antagonistic interactions between PoGV and Btk in mixed applications indicate that these agents should not be tank mixed (Sporleder, 2003).

Results of previous field tests with PoGV have been promising, though inconsistent. In Australia, Reed & Springett (1971) noted a single application of 6275 LE/ha infected 100% of fourth instars collected for several weeks following application, and that the virus could spread extensively to untreated areas, suggesting possible recycling in the crop. In the Yemeni highlands, Kroschel et al. (1996b) documented that two applications at 5×10^{13} OB/ha (5000 LE) resulted in an average mortality of 83–85% among reared larvae collected over 45 days, compared to controls. In Tunisia, four applications of PoGV applied to the soil surface at 10 000 LE/ha reduced field tuber infestation at harvest by 73% (spray formulation) or 35% (talc-based PoGV formulation), with remaining infesting populations failing to develop in storage, in either case (Ben Salah & Aalbu, 1992).

Although high dosages of PoGV may kill larvae at an early stage of development (Sporleder et al., 2004), this and previous studies indicate that for practical purposes foliar damage is expected from the generation treated, due to the slow speed of kill. The ability of potato plants to withstand significant damage without yield loss (Shields & Wyman, 1984) should limit negative impacts of defoliation, provided treatments are applied before significant infestations develop. It remains unclear what dosage of PoGV may be considered economically feasible for operational use. In our studies, reduced rate PoGV treatments were less effective than the standard rate (10^{13} OB/ha), suggesting dosage in this range is a limiting factor for control. Given the shallow dose response curve for PoGV (Sporleder et al., 2005), our data showing larval infection rates declined more by reducing dosage as opposed to application frequency was unexpected. However, the possibility that larvae were horizontally infected after field collection during rearing (possibly confounding our data) cannot be discounted.

Previous evaluations of Btk for foliar control of PTM have also produced mixed results. In Israel, Broza & Sneh (1994) reported that three applications of Btk applied at 8-day intervals controlled 69% of PTM in tomato leaves and fruit, a rate comparable with methamidophos. Kroschel (1995) reported more moderate control from five applications, 41–54% corrected mortality on foliage and 10–23% on tubers at harvest, which was attributed to excessive (10–15 days) spray intervals.

Although PoGV and Bt have received considerable interest for management of PTM, most efforts have focused on their use under rustic storage conditions, where residues are effective for extended periods (Ben Salah & Aalbu, 1992; Salama et al., 1995b; Kroschel & Koch, 1996;

Lagnaoui et al., 1997; Das et al., 1998; Hanafi, 2005). The field is considered a harsher environment for baculoviruses and Bt spores and crystals, due to the prevailing sunlight, temperature, wind, and rainfall limiting their persistence (Fuxa, 1987; Ignoffo et al., 1989). In our residue tests, a 50% decline in control (larval mortality due to treatments) occurred within 4–5 days on foliage. Studies of PoGV persistence in high altitude potato growing areas of Yemen and Peru revealed estimated half lives of 1.3 days on tubers (Kroschel et al., 1996a) and just 0.3 days on potato foliage (Sporleder, 2003). However, differences regarding location, climate, substrate, and bioassay procedures employed (e.g., latter estimates were based on inactivation of OBs rather than larval mortality) make direct comparisons between the various studies difficult.

Various methods to protect baculoviruses from ultraviolet radiation, particularly the ultraviolet B range of 280–320 nm, have been tested. Evaluations of some of the more effective techniques, such as using lignin-based spray-dried formulations (Behle et al., 2003), to prolong the insecticidal activity of PoGV in the field are warranted. Protectants that absorb in the 300–400 nm range may also improve Bt products applied on leaves (Morris, 1983; Tamez-Guerra et al., 2000). Given the high irrigation requirements of potatoes, investigation of rain-fasteners to reduce pesticide 'wash off' is also warranted.

Optimal timing will enhance the economic feasibility of using microbial pesticides. In theory, early treatment of PTM infestations is the preferred strategy. Not only are neonates the main stage exposed directly to residues (later mining stages are to some degree protected), but the lethal dosage required increases significantly with larval age (Salama et al., 1995a; Sporleder et al., 2007). Sporleder et al. (2007) estimated the LC_{50} of PoGV increased >1000-fold from neonate to 9-day-old larvae. Scouting for leaf mines (Kroschel, 1995) and/or using a temperature phenology model (Sporleder et al., 2004) will help determine the best timing of sprays, although management decisions may be limited by PTM's high dispersal ability coupled with lack of a true diapause and potential for rapid and overlapping generations, especially in tropical areas (Kroschel & Koch, 1994). Currently, the Washington State Potato Commission coordinates a trapping network throughout the Columbia Basin (eastern Washington and Oregon) in order to assist control operations and limit the spread of this pest (see http://www.potatoes.com/mapthing/plotmap1.cfm).

The effectiveness of microbial sprays may also be influenced by plant phenology. Leaf growth and expansion early in the season may rapidly dilute formulations through the production of new untreated leaf area (Ali & Young, 1993). By comparison, the increased crowding of

foliage later in the season may be expected to reduce effective spray coverage and hence initial activity of deposits. The higher foliage density later in the season coupled with the confined space within the field cages were considered to have been limiting factors with regards to our experimental treatments. Additionally, the physiological state of plants may also be important regarding PoGV infection rate. Following microscopic examination of PoGV-treated leaves, Reed (1971) suggested that OBs may enter open stomata providing a mechanism to further infect larvae mining within. Increased spray volume has been hypothesized as a method to enhance penetration of PoGV into PTM leaf tunnels (Broza & Sneh, 1994).

Gelernter & Trumble (1999) stress that microbial insecticides should be considered as a component, rather than as the sole agent, of an integrated crop management program. Integrating cultural and biological controls may further reduce the threat of economic damage. Management strategies including deep planting, frequent irrigation, and hilling up or rolling (to prevent or close cracks in the soil) and early harvest have been suggested to limit tuber infestation (Von Arx et al., 1987; Kroschel & Koch, 1994; Hanafi, 2005). In all our field cages, proportionally few tubers in the ground became infested. The well-irrigated soil and 10 cm planting depth in all probability maintained an effective barrier. Also, the additional tubers added to each cage may have arrested PTM larval movement into the soil. Crop sanitation (e.g., rotation and the destruction of volunteer plants and solanaceous weeds between seasons) may also reduce founding populations (Hanafi, 2005). In the Pacific Northwest, cull piles surrounding storage buildings facilitate larval overwintering and provide a source of moths in the spring (Jensen, 2006). Granulovirus may be compatible with native or released parasitoids of PTM (Briese, 1981; Kroschel et al., 1996b). Additional biological control agents that are being studied in our laboratory include the use of entomopathogenic nematodes applied against larvae exiting foliage late in the season (L Lacey, unpubl.) and the endophytic fungus *Muscodor albus* as a biofumigant in storage (Lacey & Neven, 2006; Lacey et al., 2008).

In conclusion, PoGV and Btk may provide a substitute for chemical insecticides to manage field infestations of PTM prior to harvest, thus reducing the risk of tuber infestations in storage. Currently, PoGV cannot be produced on artificial media and is not commercially available in USA or Europe. In order to promote interest in commercial development, further studies are needed to confirm our findings under more realistic conditions (e.g., in larger open field plots) and investigate various optimization strategies and compatibility with existing control methods. Ecological studies with PoGV to determine the between season survival in the soil and the extent of its current distribution in North America are also warranted.

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